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(54) Title: NOVEL NUCLEIC ACID AND POLYPEPTIDE

(57) Abstract

The present invention provides for nucleic acid sequences that encode novel mammalian receptor polypeptides, designated NTR–5. The invention also provides assay systems that may be used to detect and/or measure ligands that bind the MAMMALIAN NTR–5 gene product. The present invention also provides for diagnostic and therapeutic methods based on the interaction between MAMMALIAN NTR–5 and agents that initiate signal transduction through binding to MAMMALIAN NTR–5. In a specific embodiment, the MAMMALIAN NTR–5 may HUMAN NTR–5 or MOUSE NTR–5.

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NOVEL NUCLEIC ACID AND POLYPEPTIDE

Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

INTRODUCTION

The field of this invention is polypeptide molecules which regulate cell function, nucleic acid sequences encoding the polypeptides, and methods of using the nucleic acid sequences and the polypeptides. The present invention provides for novel receptor molecules, their use and assay systems useful for identifying novel ligands that interact with these receptors.

BACKGROUND OF THE INVENTION

The tumor necrosis factor receptor (TNFR) superfamily consists mostly of transmembrane proteins that elicit signal transduction in a variety of cells. Tumor necrosis factor-alpha (TNF-alpha) is a cytokine primarily produced by activated macrophages. TNF-alpha stimulates T-cell and B-cell proliferation and induces expression of adhesion molecules on endothelial cells. This cytokine also plays an important role in host defense to infection.

TNF-alpha activities are mediated through two distinct receptors, TNFR-p55 and TNFR-p75. These two receptors also mediate activities triggered by soluble lymphotoxin-alpha (LT-alpha) secreted mainly by

activated lymphocytes. Specific stimulation of TNFR-p55 induces TNF activities such as in vitro tumor cell cytotoxicity, expression of adhesion molecules on endothelial cells and keratinocytes, activation of sphingomyelinase with concomitant increases of ceramide, activation of NF-kappaB and induction of manganese superoxide dismutase mRNA. Specific stimulation of TNFR-p75 results in a proliferative response of mouse and human thymocytes and cytotoxic T-cells, fibroblasts and natural killer cells and in GM-CSF secretion in PC60 cells.

The identification of a new member of the TNFR superfamily that regulates bone resorption was recently reported. The newly identified protein was termed Osteoprotegerin (OPG) and was postulated to act as a humoral regulator of bone resorption by blocking the differentiation of osteoclasts, the cells responsible for bone resorption. (Simonet, W.S., et al., 1997, Cell 89:309-319; International Publication Number WO 97/23614 published 3 July 1997 in the name of Amgen, Inc.). However, relatively little is known about the soluble factors that act physiologically to regulate osteoclast development.

Novel receptor molecules are often identified and isolated by searching for additional members of known families of receptors using, for example, PCR-based screens or computer searches of EST databases involving known regions of homology among the family members. (See, for example, Maisonpierre, et al., 1993, Oncogene 8:1631-1637). Isolation of such so called "orphan" receptors, for which no ligand is known, and subsequent determination of the tissues in which such receptors are expressed, provides insight into the regulation of the

growth, proliferation and regeneration of cells in target tissues. Further, such receptors may be used to isolate their cognate ligands, which may then be used to regulate the survival, growth and regeneration of cells expressing the receptor. Alternatively, in the case of soluble receptors (extracellular domain only), the receptor itself can behave as a ligand.

SUMMARY OF THE INVENTION

The present invention provides for a novel mammalian receptor termed NTR-5. Specifically, the present invention provides for a novel human receptor termed HUMAN NTR-5. The present invention further provides for a novel mouse receptor termed MOUSE NTR-5. Throughout this description, reference to MAMMALIAN NTR-5 includes, but is not limited to, the specific embodiments of HUMAN NTR-5 and MOUSE NTR-5 as described herein. These receptors are related to osteoprotegerin (OPG) and to tumor necrosis factor receptor (TNFR). The present invention further provides for an isolated nucleic acid molecule encoding a MAMMALIAN NTR-5, and specifically encoding HUMAN NTR-5 or MOUSE NTR-5. Based upon its homology to osteoprotegerin, it is expected that NTR-5 will be involved in the regulation of bone mass, and may be useful for regulating development, proliferation and death of osteoblast or osteoclast cells or for regulating muscle metabolism and may be implicated in diseases or disorders of muscle.

The present invention also provides for a polypeptide that comprises the extracellular domain of MAMMALIAN NTR-5 as well as the nucleic acid which encodes such polypeptide. The invention further provides

for vectors comprising an isolated nucleic acid molecule encoding MAMMALIAN NTR-5 or its extracellular domain, which can be used to express MAMMALIAN NTR-5 or its extracellular domain in bacteria, yeast, insect or mammalian cells, preferably COS or CHO cells.

The present invention also provides for a polypeptide that comprises the intracellular domain of MAMMALIAN NTR-5 as well as the nucleic acid which encodes such polypeptide. The invention further provides for vectors comprising an isolated nucleic acid molecule encoding MAMMALIAN NTR-5 or its intracellular domain, which can be used to express MAMMALIAN NTR-5 or its intracellular domain in bacteria, yeast, insect or mammalian cells, preferably COS or CHO cells.

The invention further provides for use of the MAMMALIAN NTR-5 polypeptides comprising the extracellular and/or intracellular domain in screening for drugs that interact with MAMMALIAN NTR-5. Novel agents that bind to the polypeptides described herein may mediate survival and differentiation in cells naturally expressing polypeptides, but also may confer survival and proliferation when used to treat cells engineered to express the polypeptides. In particular embodiments, the extracellular domain of MAMMALIAN NTR-5 is utilized in screens for cognate ligands.

Preferred uses for the subject MAMMALIAN NTR-5 polypeptides include screening for agents that bind to the polypeptides. The agents may be biologically active agents (agonists), which activate the MAMMALIAN NTR-5 polypeptides or they may bind and block activation of the polypeptides (antagonists). Screening methods include incubating a

MAMMALIAN NTR-5 polypeptide in the presence of a MAMMALIAN NTR-5 polypeptide-specific binding target and a candidate agent under conditions whereby, but for the presence of the agent, the polypeptide specifically binds the binding target at a reference affinity; detecting the binding affinity of the polypeptide to the binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that the agent modulates the binding of the polypeptide to the binding target.

The invention also provides for a nucleic acid probe capable of hybridizing with a sequence included within the nucleic acid sequence encoding MAMMALIAN NTR-5 useful for the detection of NTR-5-expressing tissue in humans and animals.

The invention further provides for antibodies directed against MAMMALIAN NTR-5.

The present invention also has diagnostic and therapeutic utilities. In particular embodiments of the invention, methods of detecting aberrancies in the function or expression of the polypeptides described herein may be used in the diagnosis of disorders. In other embodiments, manipulation of the polypeptides or agonists or antagonists which bind the polypeptides may be used in the treatment of diseases. In further embodiments, the extracellular domain of the polypeptide is utilized as an agent to block the binding of a binding agent to its target.

In a further embodiment of the invention, patients who suffer from an excess of MAMMALIAN NTR-5 polypeptides may be treated by

administering an effective amount of anti-sense RNA or anti-sense oligodeoxyribonucleotides corresponding to the MAMMALIAN NTR-5 gene coding region, thereby decreasing expression of MAMMALIAN NTR-5.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides MAMMALIAN NTR-5 polypeptides which include isolated MAMMALIAN NTR-5 polypeptides and recombinant polypeptides comprising a MAMMALIAN NTR-5 amino acid sequence, or a functional MAMMALIAN NTR-5 polypeptide domain thereof having an assay-discernable MAMMALIAN NTR-5-specific activity. Accordingly, the polypeptides may be deletion mutants of the disclosed MAMMALIAN NTR-5 polypeptide and may be provided as fusion products, e.g., with non-MAMMALIAN NTR-5 polypeptides. The subject MAMMALIAN NTR-5 polypeptides have MAMMALIAN NTR-5-specific activity or function.

A number of applications for MAMMALIAN NTR-5 polypeptides are suggested from their properties. MAMMALIAN NTR-5 polypeptides may be useful in the study and treatment of conditions similar to those which are treated using TNF. Furthermore, the MAMMALIAN NTR-5 cDNA may be useful as a diagnostic tool, such as through the use of oligonucleotides as primers in a PCR test to amplify those sequences having similarities to the oligonucleotide primer, and to see how much MAMMALIAN NTR-5 mRNA is present in a particular tissue or sample. The isolation of MAMMALIAN NTR-5, of course, also provides the key to isolate its putative ligand, other MAMMALIAN NTR-5 binding polypeptides, and/or study its properties.

MAMMALIAN NTR-5-specific activity or function may be determined by convenient in vitro, cell based or in vivo assays. In vitro or cell based assays include but are not limited to binding assays and cell culture assays. In vivo assays include but are not limited to immune response, gene therapy and transgenic animals. Binding assays encompass any assay where the specific molecular interaction of a MAMMALIAN NTR-5 polypeptide with a binding target is evaluated. The binding target may be a natural binding target, or a nonnatural binding target such as a specific immune polypeptide such as an antibody, or a MAMMALIAN NTR-5-specific binding agent.

The claimed MAMMALIAN NTR-5 polypeptides may be isolated or pure - an "isolated" polypeptide is one that is no longer accompanied by some of the material with which it is associated in its natural state, and that preferably constitutes at least about 0.5%, and more preferably at least about 5% by weight of the total polypeptide in a given sample; a "pure" polypeptide constitutes at least about 90%, and preferably at least about 99% by weight of the total polypeptide in a given sample. The subject polypeptides may be synthesized, produced by recombinant technology, or purified from cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the subject compositions, see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY).

The subject polypeptides find a wide variety of uses including but not limited to use as immunogens, targets in screening assays, bioactive reagents for modulating cell growth, differentiation and/or function. For example, the invention provides methods for modifying the physiology of a cell comprising contacting the extracellular surface of the cell or medium surrounding the cell with an exogenous MAMMALIAN NTR-5 polypeptide under conditions whereby the added polypeptide specifically interacts with a component of the medium and/or the extracellular surface to effect a change in the physiology of the cell. According to these methods, the extracellular surface includes plasma membrane-associated molecules. The term "exogenous MAMMALIAN NTR-5 polypeptide" refers to polypeptides not made by the cell or, if so, expressed at non-natural levels, times or physiologic locales. Media, include, but not limited to, in vitro culture media and/or physiological fluids such as blood, synovial fluid and lymph. The polypeptides may be introduced, expressed, or repressed in specific populations of cells by any convenient way, including but not limited to, microinjection, promoter-specific expression of recombinant protein or targeted delivery of lipid vesicles.

The invention provides MAMMALIAN NTR-5-specific binding agents, methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. MAMMALIAN NTR-5-specific binding agents include MAMMALIAN NTR-5-specific antibodies (See, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and also includes other binding agents identified with assays such as one-, two- and three-hybrid screens, and non-natural binding agents identified in

screens of chemical libraries such as described below. Agents of particular interest modulate MAMMALIAN NTR-5 polypeptide function.

The invention provides MAMMALIAN NTR-5 nucleic acids, which find a wide variety of applications, including but not limited to, use as translatable transcripts, hybridization probes, PCR primers, or diagnostic nucleic acids, as well as use in detecting the presence of MAMMALIAN NTR-5 genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional MAMMALIAN NTR-5 homologs and structural analogs.

The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i.e., no longer accompanied by some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, more preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to a nucleotide(s) other than that to which it is joined on a natural chromosome. Nucleic acids comprising the nucleotide sequence disclosed herein and fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by a sequence other than that to which it is joined on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, which is immediately flanked by a sequence other than that to which it is joined on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide, example, modified stability.

The sequence of the disclosed MAMMALIAN NTR-5 nucleic acid is used to obtain the deduced MAMMALIAN NTR-5 polypeptide sequence. Further, the sequence of the disclosed MAMMALIAN NTR-5 nucleic acid is optimized for selected expression systems (Holler, et al., (1993) Gene 136:323-328; Martin, et al., (1995) Gene 154:150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural MAMMALIAN NTR-5 encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc., Madison, WI). MAMMALIAN NTR-5 encoding nucleic acids may be part of expression vectors and may be incorporated into recombinant host cells, e.g., for expression and screening, for transgenic animals, or for functional studies such as the efficacy of candidate drugs for diseases associated with MAMMALIAN NTR-5 polypeptide-mediated signal transduction. Expression systems are selected and/or tailored to effect MAMMALIAN NTR-5 polypeptide structural and functional variants through alternative post-translational processing.

The invention also provides for nucleic acid hybridization probes and replication/amplification primers having a MAMMALIAN NTR-5 cDNA specific sequence and sufficient to effect specific hybridization with SEQ. NO. 1 or SEQ. NO. 3. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5x SSPE (0.18 M NaCl, 0.01 M $NaPO_4$, pH 7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at

42°C with 0.2x SSPE buffer at 42°C. MAMMALIAN NTR-5 cDNA homologs can also be distinguished from one another using alignment algorithms, such as BLASTX (Altschul, et al., (1990) Basic Local Alignment Search Tool, J. Mol. Biol. 215:403-410).

MAMMALIAN NTR-5 hybridization probes find use in identifying wild-type and mutant alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. MAMMALIAN NTR-5 nucleic acids are also used to modulate cellular expression or intracellular concentration or availability of active MAMMALIAN NTR-5 polypeptides. MAMMALIAN NTR-5 inhibitory nucleic acids are typically antisense- single stranded sequences comprising complements of the disclosed MAMMALIAN NTR-5 coding sequences. Antisense modulation of the expression of a given MAMMALIAN NTR-5 polypeptide may employ antisense nucleic acids operably linked to gene regulatory sequences. Cells are transfected with a vector comprising a MAMMALIAN NTR-5 sequence with a promoter sequence oriented such that transcription of the gene yields an antisense transcript capable of binding to endogenous MAMMALIAN NTR-5 encoding mRNA. Transcription of the antisense nucleic acid may be constitutive or inducible and the vector may provide for stable extrachromosomal maintenance or integration. Alternatively, single-stranded antisense nucleic acids that bind to genomic DNA or mRNA encoding a given MAMMALIAN NTR-5 polypeptide may be administered to the target cell, in or temporarily isolated from a host, at a concentration that results in a substantial reduction in expression of the targeted polypeptide. An enhancement in MAMMALIAN NTR-5 expression is effected by introducing into the targeted cell type

MAMMALIAN NTR-5 nucleic acids which increase the functional expression of the corresponding gene products. Such nucleic acids may be MAMMALIAN NTR-5 expression vectors, vectors which upregulate the functional expression of an endogenous allele, or replacement vectors for targeted correction of mutant alleles. Techniques for introducing the nucleic acids into viable cells are known in the art and include, but are not limited to, retroviral-based transfection or viral coat protein-liposome mediated transfection.

The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of MAMMALIAN NTR-5 modulatable cellular function. Generally, these screening methods involve assaying for compounds which modulate the interaction of MAMMALIAN NTR-5 with a natural MAMMALIAN NTR-5 binding target. A wide variety of assays for binding agents are provided including, but not limited to, protein-protein binding assays, immunoassays, or cell based assays. Preferred methods are amenable to automated, cost-effective, high throughput screening of chemical libraries for lead compounds.

In vitro binding assays employ a mixture of components including a MAMMALIAN NTR-5 polypeptide, which may be part of a fusion product with another peptide or polypeptide, e.g., a tag for detection or anchoring. The assay mixtures comprise a natural MAMMALIAN NTR-5 binding target. While native binding targets may be used, it is frequently preferred to use portions thereof as long as the portion provides binding affinity and avidity to the subject MAMMALIAN NTR-5 conveniently measurable in the assay. The assay mixture also

comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds, preferably small organic compounds, and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents such as salts, buffers, neutral proteins, e.g., albumin, detergents, protease inhibitors, nuclease inhibitors, or antimicrobial agents may also be included. The mixture components can be added in any order that provides for the requisite bindings and incubations may be performed at any temperature which facilitates optimal binding. The mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the MAMMALIAN NTR-5 polypeptide specifically binds the binding target, portion or analog with a reference binding affinity. Incubation periods are chosen for optimal binding but are also minimized to facilitate rapid, high throughput screening.

After incubation, the agent-biased binding between the MAMMALIAN NTR-5 polypeptide and one or more binding targets is detected by any convenient way. For cell-free binding type assays, a separation step is often used to separate bound from unbound components. Separation may be effected by any number of methods that include, but are not limited to, precipitation or immobilization followed by washing by, e.g., membrane filtration or gel chromatography. For cell-free binding assays, one of the components usually comprises or is coupled to a label. The label may provide for direct detection as radioactivity, luminescence, optical or electron density, or indirect detection such as an epitope tag or an enzyme. A variety of methods may be used to detect the label depending on the nature of the label and other assay

components, including but not limited to, through optical or electron density, radiative emissions, nonradiative energy transfers, or indirectly detected with, as a nonlimiting example, antibody conjugates. A difference in the binding affinity of the MAMMALIAN NTR-5 polypeptide to the target in the absence of the agent as compared with the binding affinity in the presence of the agent indicates that the agent modulates the binding of the MAMMALIAN NTR-5 polypeptide to the corresponding binding target. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least a 90% difference.

The invention provides for a method for modifying the physiology of a cell comprising an extracellular surface in contact with a medium, said method comprising the step of contacting said medium with an exogenous MAMMALIAN NTR-5 polypeptide under conditions whereby said polypeptide specifically interacts with at least one of the components of said medium to effect a change in the physiology of said cell.

The invention further provides for a method for screening for biologically active agents, said method comprising the steps of a) incubating a MAMMALIAN NTR-5 polypeptide in the presence of a MAMMALIAN NTR-5 polypeptide-specific binding target and a candidate agent, under conditions whereby, but for the presence of said agent, said polypeptide specifically binds said binding target at a reference affinity; b) detecting the binding affinity of said polypeptide to said binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity

indicates that said agent modulates the binding of said polypeptide to said binding target.

One embodiment of the invention is an isolated MAMMALIAN NTR-5 polypeptide comprising the amino acid sequence as set forth herein or a fragment thereof having MAMMALIAN NTR-5-specific activity.

Another embodiment of the invention is a recombinant nucleic acid encoding MAMMALIAN NTR-5 polypeptide comprising the amino acid sequence as set forth herein or a fragment thereof having MAMMALIAN NTR-5-specific activity.

Still another embodiment is an isolated nucleic acid comprising a nucleotide sequence as set forth herein in SEQ. NO. 3 or a fragment thereof having at least 18 consecutive bases and which can specifically hybridize with a nucleic acid having the sequence of native MAMMALIAN NTR-5.

The present invention also provides for antibodies to the MAMMALIAN NTR-5 polypeptides described herein which are useful for detection of the polypeptides in, for example, diagnostic applications. For preparation of monoclonal antibodies directed toward MAMMALIAN NTR-5 polypeptides, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human

monoclonal antibodies (Cole et al., 1985, in "Monoclonal Antibodies and Cancer Therapy", Alan R. Liss, Inc. pp. 77-96) and the like are within the scope of the present invention.

The monoclonal antibodies for diagnostic or therapeutic use may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7308-7312; Kozbor et al., 1983, Immunology Today 4:72-79; Olsson et al., 1982, Meth. Enzymol. 92:3-16). Chimeric antibody molecules may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851, Takeda et al., 1985, Nature 314:452).

Various procedures known in the art may be used for the production of polyclonal antibodies to the MAMMALIAN NTR-5 polypeptides described herein. For the production of antibody, various host animals can be immunized by injection with the MAMMALIAN NTR-5 polypeptides, or fragments or derivatives thereof, including but not limited to rabbits, mice and rats. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum.

A molecular clone of an antibody to a selected MAMMALIAN NTR-5 polypeptide epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or antigen binding region thereof.

The present invention provides for antibody molecules as well as fragments of such antibody molecules. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Antibody molecules may be purified by known techniques including, but not limited to, immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof.

The following example is offered by way of illustration and not by way of limitation.

EXAMPLE I - CLONING AND SEQUENCING OF THE HUMAN NTR-5 GENE

Amino acid sequences of known human and mouse members of the TNF family were used as tblastn queries to search the NIH EST database of

random fragments of mRNA sequences (Altschul et al., (1990), Basic local alignment search tool, J. Mol. Biol. 215:403-10). Each query generated a list of hits, i.e. EST sequences with a substantial sequence similarity to the query sequence. Typically, the hits on top of the list corresponded to mRNA copies of the query protein, followed by ESTs derived from other members of the family and random-chance similarities.

A parser program was used to combine and sort all the hits from searches with all the members of the family. This allowed rapid subtraction of all the hits corresponding to known proteins. The remaining hits were analyzed for conservation of sequence motifs characteristic for the family. Additional database searches were performed to identify overlapping ESTs. Two mouse cDNA clones from the I.M.A.G.E. consortium were discerned to contain homologous sequence. Clone # 472300 (the '300 clone) (GeneBank Accession No. AA036247) and clone #427152 (the '152 clone) (GeneBank Accession No. AA003356) were obtained from Research Genetics, Inc. (Huntsville, AL) and sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA).

Both the '300 clone and the '152 clone contained a single coding frame encoding a 215 amino acid protein (SEQ. NO. 2) designated MOUSE NTR-5 as set forth below. MOUSE NTR-5 revealed sequence similarity to members of the TNF receptor family.

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                    10          20          30          40          50          60
                     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
SEQ. NO. 1 -CT GGC ATG GAG TTG TCC AAG GAA TGT GGC TTC GGC TAT GGG GAG GAT GCA CAG TGT GTG
SEQ. NO. 2    Gly Met Glu Leu Ser Lys Glu Cys Gly Phe Gly Tyr Gly Glu Asp Ala Gln Cys Val>

                    70          80          90          100         110         120
                     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CCC TGC AGG CCG CAC CGG TTC AAG GAA GAC TGG GGT TTC CAG AAG TGT AAG CCA TGT GCG
Pro Cys Arg Pro His Arg Phe Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Ala>

                    130         140         150         160         170         180
                     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GAC TGT GCG CTG GTG AAC CGC TTT CAG AGG GCC AAC TGC TCA CAC ACC AGT GAT GCT GTC
Asp Cys Ala Leu Val Asn Arg Phe Gln Arg Ala Asn Cys Ser His Thr Ser Asp Ala Val>

                    190         200         210         220         230         240
                     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGC GGG GAC TGC CTG CCA GGA TTT TAC CGG AAG ACC AAA CTG GTT GGT TTT CAA GAC ATG
Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val Gly Phe Gln Asp Met>

                    250         260         270         280         290         300
                     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GAG TGT GTG CCC TGC GGA GAC CCA CCT CCT CCC TAC GAA CCA CAC TGT ACC AGC AAG GTG
Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro Tyr Glu Pro His Cys Thr Ser Lys Val>

                    310         320         330         340         350         360
                     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAC CTT GTG AAG ATT TCC TCC ACC GTC TCC AGC CCT CGG GAC ACG GCG CTG GCT GCC GTC
Asn Leu Val Lys Ile Ser Ser Thr Val Ser Ser Pro Arg Asp Thr Ala Leu Ala Ala Val>

                    370         380         390         400         410         420
                     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
ATC TGC AGT GCT CTG GCC ACG GTG CTG CTC GCC CTG CTC ATC CTG TGT GTC ATC TAC TGC
Ile Cys Ser Ala Leu Ala Thr Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr Cys>

                    430         440         450         460         470         480
                     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAG AGG CAG TTC ATG GAG AAG AAA CCC AGC TGT AAG CTC CCA TCC CTC TGT CTC ACT GTG
Lys Arg Gln Phe Met Glu Lys Lys Pro Ser Cys Lys Leu Pro Ser Leu Cys Leu Thr Val>

                    490         500         510         520         530
                     *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAG TGA GCT TGT TAG CAT TGT CAC CCA AGA GTT CTC AAG ACA CCT GGC TGA GAC CTA AG
Lys ***>
```

The MOUSE NTR-5 cDNA as set forth in SEQ. NO. 1 above was used as a probe to screen Northern blots of human mRNA derived from heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. Detectable expression levels of HUMAN NTR-5 were present in heart and

kidney. A human heart cDNA library (Stratagene Cat. # 936208) was plated in six 243 mm x 243 mm plates at 2.5 x 10^5 plaques per plate, transferred to nitrocellulose using standard techniques known in the art, and probed with the MOUSE NTR-5 probe. The nitrocellulose filters were prehybridized for 30 minutes at 65°C in 155 ml of hybridization buffer containing 0.5 M $NaPO_4$ (pH 7), 1% bovine serum albumin (Fraction V, Sigma), 7% SDS, 1 mM EDTA and 100 ng/ml sonicated, denatured salmon sperm DNA. The ^{32}P-ATP-radiolabeled MOUSE NTR-5 probe was added to the prehybridization mixture and hybridization was carried out at 60°C overnight. The filters were washed with 2x SSC several times at room temperature and for 30 minutes at 60°C and exposed to x-ray film overnight at -80°C. Two positive clones were obtained based on their ability to cross-hybridize with the MOUSE NTR-5 probe. One of these positive clones, human clone #2, was amplified by PCR using oligonucleotides specific to the T7/T3 vector components and the resultant PCR-amplified DNA fragment was sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). The human clone #2 sequence contained an open reading frame encoding a 328 amino acid protein with sequence similarity to other members of the TNF receptor family, including features characteristic of membrane attached proteins predictive of a signal peptide sequence at amino acids 1-29, an extracellular domain at amino acids 30-169, a transmembrane motif at amino acids 170-193, and an intracellular domain at amino acids 194-328 (see SEQ. NO. 4).

The nucleotide and deduced amino acid sequence of human clone #2 is set forth below.

```
                10          20          30          40          50          60
                 *           *           *           *           *           *
SEQ. NO. 3  ATG GCT TTA AAA GTG CTA CTA GAA CAA GAG AAA ACG TTT TTC ACT CTT TTA GTA TTA CTA
SEQ. NO. 4  Met Ala Leu Lys Val Leu Leu Glu Gln Glu Lys Thr Phe Phe Thr Leu Leu Val Leu Leu>

                70          80          90          100         110         120
                 *           *           *           *           *           *
GGC TAT TTG TCA TGT AAA GTG ACT TGT GAA TCA GGA GAC TGT AGA CAG CAA GAA TTC AGG
Gly Tyr Leu Ser Cys Lys Val Thr Cys Glu Ser Gly Asp Cys Arg Gln Gln Glu Phe Arg>

               130         140         150         160         170         180
                 *           *           *           *           *           *
GAT CGG TCT GGA AAC TGT GTT CCC TGC AAC CAG TGT GGG CCA GGC ATG GAG TTG TCT AAG
Asp Arg Ser Gly Asn Cys Val Pro Cys Asn Gln Cys Gly Pro Gly Met Glu Leu Ser Lys>

               190         200         210         220         230         240
                 *           *           *           *           *           *
GAA TGT GGC TTC GGC TAT GGG GAG GAT GCA CAG TGT GTG ACG TGC CGG CTG CAC AGG TTC
Glu Cys Gly Phe Gly Tyr Gly Glu Asp Ala Gln Cys Val Thr Cys Arg Leu His Arg Phe>

               250         260         270         280         290         300
                 *           *           *           *           *           *
AAG GAG GAC TGG GGC TTC CAG AAA TGC AAG CCC TGT CTG GAC TGC GCA GTG GTG AAC CGC
Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Leu Asp Cys Ala Val Val Asn Arg>

               310         320         330         340         350         360
                 *           *           *           *           *           *
TTT CAG AAG GCA AAT TGT TCA GCC ACC AGT GAT GCC ATC TGC GGG GAC TGC TTG CCA GGA
Phe Gln Lys Ala Asn Cys Ser Ala Thr Ser Asp Ala Ile Cys Gly Asp Cys Leu Pro Gly>

               370         380         390         400         410         420
                 *           *           *           *           *           *
TTT TAT AGG AAG ACG AAA CTT GTC GGC TTT CAA GAC ATG GAG TGT GTG CCT TGT GGA GAC
Phe Tyr Arg Lys Thr Lys Leu Val Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp>

               430         440         450         460         470         480
                 *           *           *           *           *           *
CCT CCT CCT CCT TAC GAA CCG CAC TGT GCC AGC AAG GTC AAC CTC GTG AAG ATC GCG TCC
Pro Pro Pro Pro Tyr Glu Pro His Cys Ala Ser Lys Val Asn Leu Val Lys Ile Ala Ser>

               490         500         510         520         530         540
                 *           *           *           *           *           *
ACG GCC TCC AGC CCA CGG GAC ACG GCG CTG GCT GCC GTT ATC TGC AGC GCT CTG GCC ACC
Thr Ala Ser Ser Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser Ala Leu Ala Thr>

               550         560         570         580         590         600
                 *           *           *           *           *           *
GTC CTG CTG GCC CTG CTC ATC CTC TGT GTC ATC TAT TGT AAG AGA CAG TTT ATG GAG AAG
Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr Cys Lys Arg Gln Phe Met Glu Lys>

               610         620         630         640         650         660
                 *           *           *           *           *           *
AAA CCC AGC TGG TCT CTG CGG TCG CAG GAC ATT CAG TAC AAC GGC TCT GAG CTG TCG TGT
Lys Pro Ser Trp Ser Leu Arg Ser Gln Asp Ile Gln Tyr Asn Gly Ser Glu Leu Ser Cys>
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          670         680         690         700         710         720
           *           *           *           *           *           *
TTT GAC AGA CCT CAG CTC CAC GAA TAT GCC CAC AGA GCC TGC TGC CAG TGC CGC CGT GAC
Phe Asp Arg Pro Gln Leu His Glu Tyr Ala His Arg Ala Cys Cys Gln Cys Arg Arg Asp>

          730         740         750         760         770         780
           *           *           *           *           *           *
TCA GTG CAG ACC TGC GGG CCG GTG CGC TTG CTC CCA TCC ATG TGC TGT GAG GAG GCC TGC
Ser Val Gln Thr Cys Gly Pro Val Arg Leu Leu Pro Ser Met Cys Cys Glu Glu Ala Cys>

          790         800         810         820         830         840
           *           *           *           *           *           *
AGC CCC AAC CCG GCG ACT CTT GGT TGT GGG GTG CAT TCT GCA GCC AGT CTT CAG GCA AGG
Ser Pro Asn Pro Ala Thr Leu Gly Cys Gly Val His Ser Ala Ala Ser Leu Gln Ala Arg>

          850         860         870         880         890         900
           *           *           *           *           *           *
AAG CTT AAA GAA CCT GCT TCT TTC TGC AGT AGA AGC GTG TGC TGG AAC CCA AAG AGT ACT
Lys Leu Lys Glu Pro Ala Ser Phe Cys Ser Arg Ser Val Cys Trp Asn Pro Lys Ser Thr>

          910         920         930         940         950         960
           *           *           *           *           *           *
CCT TTG TTA GGC TTA TGG ACT GAG CAG TCT GGA CCT TGC ATG GCT TCT GGG GCA AAA ATA
Pro Leu Leu Gly Leu Trp Thr Glu Gln Ser Gly Pro Cys Met Ala Ser Gly Ala Lys Ile>

          970         980
           *           *
AAT CTG AAC CAA ACT GAC GGC ATT TGA
Asn Leu Asn Gln Thr Asp Gly Ile ***>
```

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding HUMAN NTR-5.

2. An isolated nucleic acid molecule according to claim 1, having a sequence selected from the group consisting of:
   (a) the nucleotide sequence comprising the coding region of the HUMAN NTR-5 as set forth in SEQ. NO. 3;
   (b) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of (a) and which encodes a molecule having the biological activity of the HUMAN NTR-5; or
   (c) a nucleotide sequence which, but for the degeneracy of the genetic code would hybridize to a nucleotide sequence of (a) or (b), and which encodes a molecule having the biological activity of the HUMAN NTR-5.

3. A vector which comprises a nucleic acid molecule of claim 1 or 2.

4. A vector according to claim 3, wherein the nucleic acid molecule Is operatively linked to an expression control sequence capable of directing its expression in a host cell.

5. An isolated nucleic acid molecule encoding a MAMMALIAN NTR-5.

6. Isolated MAMMALIAN NTR-5 polypeptide.

7. Isolated HUMAN NTR-5 polypeptide encoded by the nucleic acid molecule of claim 2.

8. A host-vector system for the production of HUMAN NTR-5 polypeptide which comprises a vector of claim 3 or 4, in a host cell.

9. A host-vector system according to claim 8, wherein the host cell is a bacterial, yeast, insect or mammalian cell.

10. A method of producing HUMAN NTR-5 polypeptide which comprises growing cells of a host-vector system of claim 8 or 9, under conditions permitting the production of HUMAN NTR-5 polypeptide and recovering the HUMAN NTR-5 polypeptide so produced.

11. An antibody which specifically binds NTR-5 polypeptide of claim 6 or 7.

12. An antibody according to claim 11, which is a monoclonal antibody.

13. A composition comprising HUMAN NTR-5 polypeptide according to claim 7 and a carrier.

14. A composition comprising an antibody according to claim 11 or 12, and a carrier.

15. HUMAN NTR-5 polypeptide according to claim 7, an antibody according to claim 11 or 12, or a composition according to claim 13 or 14, for use in a method of treatment of a human or animal body, or in a method of diagnosis.

16. A polypeptide produced by the method of claim 10.

17. A polypeptide comprising the extracellular portion of the MAMMALIAN NTR-5 polypeptide fused to an immunoglobulin constant region.

18. The polypeptide of claim 17, wherein the constant region is the human immunoglobulin gamma-1 constant region.

19. A polypeptide comprising the extracellular portion of the MAMMALIAN NTR-5 polypeptide fused to an immunoglobulin Fc region.

20. The polypeptide of claim 19, wherein the Fc region is the human immunoglobulin gamma-1 Fc region.